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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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☐ *Additional inventors are being named on the _______ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

METHOD AND APPARATUS FOR DIAGNOSING NEOVASCULARIZED TISSUES

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS ***(check all that apply)***

☑ Specification *Number of Pages* 22

☑ Drawing(s) *Number of Sheets* 2

☐ Application Data Sheet. See 37 CFR 1.76

☐ CD(s), Number

☑ Other (specify) Patent Postcard

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.

☑ A check or money order is enclosed to cover the filing fees

☑ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 50-1212/UTSJ:041USP1/LVP

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☑ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: ____________

Respectfully submitted,

SIGNATURE [signature] Date 01/23/2003

TYPED or PRINTED NAME Luiz Von-Paumgartten

REGISTRATION NO. *(if appropriate)* 52,330

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Docket Number: UTSJ:041USP1

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

PATENT
UTSJ:041USP1

PROVISIONAL APPLICATION FOR UNITED STATES LETTERS PATENT

for

METHOD AND APPARATUS FOR DIAGNOSING NEOVASCULARIZED TISSUES

by

Dhiraj Sardar
and
Andrew Tsin

EXPRESS MAIL MAILING LABEL
NUMBER EV 119098826 US
DATE OF DEPOSIT January 23, 2003

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY-SPONSORED RESEARCH AND DEVELOPMENT

This invention was made with support from the National Science Foundation and the National Institutes of Health. The Government may have certain rights in this invention.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention relates generally to the field of medical diagnostics. More particularly, the invention relates to the diagnosing of neovascularized tissue.

2. Discussion of the Related Art

In recent years, there has been a considerable interest in the investigation of ocular neovascularization. Ocular neovascularization is the formation of new blood vessels in the development of diseases such as, for example, macular degeneration and diabetic retinopathy.

Retinal neovascularization resulting from diabetic retinopathy is the most common cause of blindness in young patients in major industrialized countries, and choroidal neovascularization resulting from age-related macular degeneration is the most common cause of severe vision loss in elderly patients.

During neovascularization, increased amounts of blood in capillaries change the optical properties of the tissue. Meanwhile, it has been known that ocular tissues are inherently bi-refringent and may alter the polarization of incident light in scattering events according to the their geometry and optical properties.

What is needed is a non-invasive method and apparatus for detecting neovascularized

ocular tissues. What is also needed is a non-invasive method and apparatus for diagnosing ocular diseases such as diabetic retinopathy and macular degeneration.

SUMMARY OF THE INVENTION

There is a need for the following embodiments. Of course, the invention is not limited to these embodiments.

According to an aspect of the invention, a method for diagnosing an ocular disease includes placing an ocular tissue in the path of a light beam, measuring a polarization shift of the light beam, and diagnosing an ocular disease if the measured polarization shift corresponds to a polarization shift of a neovascularized tissue.

According to another aspect of the invention, an apparatus for diagnosing an ocular disease includes: a laser, a polarizer coupled to the laser, a tissue sample holder coupled to the polarizer, the tissue sample holder having an ocular tissue sample, an analyzer coupled to the tissue sample holder, a detector coupled to the analyzer, and a data acquisition system coupled to the detector, the data acquisition system measuring a polarization shift of the light beam and diagnosing an ocular disease if the measured polarization shift corresponds to a polarization shift of a neovascularized tissue.

These, and other, embodiments of the invention will be better appreciated and understood when considered in conjunction with the following description and the accompanying drawings. It should be understood, however, that the following description, while indicating various embodiments of the invention and numerous specific details thereof,

is given by way of illustration and not of limitation. Many substitutions, modifications, additions and/or rearrangements may be made within the scope of the invention without departing from the spirit thereof, and the invention includes all such substitutions, modifications, additions and/or rearrangements.

BRIEF DESCRIPTION OF THE DRAWINGS

The drawings accompanying and forming part of this specification are included to depict certain aspects of the invention. A clearer conception of the invention, and of the components and operation of systems provided with the invention, will become more readily apparent by referring to the exemplary, and therefore non-limiting, embodiments illustrated in the drawings, wherein like reference numerals (if they occur in more than one view) designate the same or similar elements. The invention may be better understood by reference to one or more of these drawings in combination with the description presented herein. It should be noted that the features illustrated in the drawings are not necessarily drawn to scale.

FIG. 1 is a diagram of a setup for polarization measurements of a retinal tissue, representing an embodiment of the invention.

FIG. 2 is a diagram of a setup for polarization measurements of a retinal pigment epithelium (RPE)/choroidal tissue, representing an embodiment of the invention.

DESCRIPTION OF PREFERRED EMBODIMENTS

The invention and the various features and advantageous details thereof are explained more fully with reference to the non-limiting embodiments that are illustrated in the accompanying drawings and detailed in the following description. Descriptions of well known starting materials, processing techniques, components and equipment are omitted so as not to unnecessarily obscure the invention in detail. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only and not by way of limitation. Various substitutions, modifications, additions and/or rearrangements within the spirit and/or scope of the underlying inventive concept will become apparent to one of ordinary skill in the art from this disclosure.

The invention may include a method and/or apparatus for determining an optical property of an ocular tissue. The invention may also include a method and/or apparatus for detecting neovascularized ocular tissues and diagnosing medical conditions or diseases. Examples of ocular neovascularized tissue may include but are not limited to: diabetic retinal tissue, choroidal capillaries, and tumor tissues.

The invention may include a method for relating the optical properties of a biological tissue to its constituents. During neovascularization, increased amounts of blood in capillaries may change the optical properties of the tissue. For example, retinal vascular development may occur due to a combination of vasculogenesis and angiogenesis. Retinal and retinal pigment epithelium (RPE)/choroidal vessels may multiply with increased amounts of blood in

the capillaries, thereby enhancing their scattering, intensity change, and polarization shift properties.

In one embodiment, the invention may include using a probing light or laser which may be linearly polarized at various angles, such as, for example: right circularly, left circularly, or elliptically polarized. Light reflected from (or transmitted through) the tissue may be analyzed. An analytical method may be used characterize the optical properties of the tissue, such as the one demonstrated by the Stokes-vector Mueller-matrix approach to polarization and light scattering.

The invention may include a non-invasive method and/or apparatus for the early detection and diagnosis of ocular diseases associated with neovascularization processes, such as diabetic retinopathy and macular degeneration. According to one aspect of the invention, neovascularized ocular tissues may exhibit significantly different optical properties (such as higher scattering and increased degree of polarization shifts of the backscattered polarized light) than healthy tissues. According to another aspect of the invention, these differences may be quantitatively accessed at different pathological stages using tissue polarimetry.

Referring to **FIG. 1**, a diagram of a setup **100** for polarization measurements for a retinal tissue is depicted, according to one embodiment of the invention. A laser **105** is coupled to a first polarizer **110**. The first polarizer **110** is coupled to a sample holder **115** comprising retinal tissue. The sample holder **115** is coupled to a second polarizer/analyzer **120**. The analyzer **120** is coupled to a detector **125**. The detector **125** is coupled to a data acquisition system **130**. The laser **105**, the detector **125**, and the data acquisition system **130** may each be coupled to a power supply (not shown).

In one embodiment, the laser **105** may be a He-Ne laser (such as the 1101P laser by Uniphase Corporation) with a power of 4 milliwatt and beam diameter of 3 mm. The laser **105** creates a laser beam that is passed through the linear polarizer **110**, the retinal tissue in the sample holder **115**, and the analyzer **120**. The polarizers/analyzers **110**, **120** may be, for example, the 25010 polarizers from Oriel Corporation. The detector **125** may be a photodiode detector which is coupled to a power supply such as, for example, a Cenco model 31382 supply (not shown). The detector **125** is connected to the data acquisition system **130**, which may be, for example, a Fluke model 77 series II multimeter. The data acquisition system **130** may be a meter, a digital meter, a data aqcuisition system, a computer, or the like. The data acquisition system **130** may measure, for example, a polarization shift and/or an intensity variation.

In a first step of a data acquisition operation, the sample holder **115** and the analyzer **120** are absent from the setup **100**, and the polarizer **110** is rotated until the maximum beam intensity is obtained, indicating that the beam is completely polarized. Once the maximum laser intensity is achieved, the analyzer **120** may be placed between the photodiode **125** and the polarizer **110** (still without the sample **115** in the light path). The analyzer **120** is rotated to maximize the light intensity so that that the transmission axes of the polarizer **110** and the analyzer **120** are parallel with respect to each other. Next, the sample holder **115** containing a retinal tissue may be placed between the polarizer **110** and the analyzer **120**, causing the polarization plane to shift due to the anisotropic property of the tissues. The polarization shift of the scattered laser light may be observed and the shift may be determined by rotating the analyzer **120** until maximum light intensity is measured with the data acquisition system **130**. The data acquisition system **130** may take laser polarization and/or laser intensity measurements corresponding to different locations on

the retinal tissue and process the acquired data. In one embodiment, an average of three measurements is taken for each sample location.

Due to the opacity of RPE/choroidal tissue, laser beams may not be able to penetrate RPE/choroidal tissue samples. In one embodiment, a modification of the experimental setup **100** detailed in **FIG. 1** may be made for measurements of the polarized light scattered off a RPE/choroidal tissue sample.

Referring to **FIG. 2**, a diagram of an experimental setup **200** for polarization measurements of a RPE/choroidal tissue is depicted, according to one embodiment of the invention. A clean glass slide (not shown) may be placed in the sample holder **125**, and the scattered beam may be directed at approximately a right angle with respect to the direction of the incident laser beam. The analyzer **120** and the photodiode **110** may be aligned with the direction of the most intense scattered beam, and the same technique as described above may be employed to assure that the transmission axes of both the polarizer **110** and the analyzer **120** are parallel with respect to each other. The glass slide may then be replaced by the RPE/choroidal tissue. The polarization shift for the RPE/choroid tissue may be determined in the same manner as retinal tissue of **FIG. 1**. The data acquisition system **130** may take laser polarization and/or laser intensity measurements corresponding to different locations on the RPE/choroid tissue and process the acquired data. In one embodiment, an average of three measurements is taken for each sample location.

The invention may include using an experimental methodology as described above for performing polarization measurements on retinal and RPE/choroidal tissues placed together

(combination retinal and RPE/choroidal tissues). In this case, a retinal tissue sample may be placed in front of the RPE/choroidal tissue sample.

EXAMPLES

Specific embodiments of the invention will now be further described by the following, nonlimiting examples which will serve to illustrate in some detail various features. The following examples are included to facilitate an understanding of ways in which the invention may be practiced. It should be appreciated that the examples which follow represent embodiments discovered to function well in the practice of the invention, and thus can be considered to constitute preferred modes for the practice of the invention. However, it should be appreciated that many changes can be made in the exemplary embodiments which are disclosed while still obtaining like or similar result without departing from the spirit and scope of the invention. Accordingly, the examples should not be construed as limiting the scope of the invention.

Example 1

Samples of bovine ocular (retina and RPE/choroid) tissues were prepared from fresh eyes obtained from a slaughter plant and preserved at 0°C during transportation for 45 min. Upon arrival in the laboratory, anterior segments including cornea, lense, and aqueous vitreous humor fluid were removed from the eyes. Next, the retina was carefully lifted from the posterior eye cup and mounted between two glass slides. The RPE/choroid was subsequently removed from the eye and similarly mounted. The thickness of the retinal and RPE/choroidal tissues were

approximately 0.15 and 0.10 mm, respectively. A small amount of vacuum grease was applied to the edges of the glass slides in order to maintain the moisture of the sample. All data was collected at room temperature within two hours from the slaughter of the animals.

The data acquisition operation described herein was performed, and polarization shift measurements of both the retinal and RPE/choroidal tissues taken from the bovine left and right eyes are given in Table I.

Table I

Polarization shift (in degrees) in the bovine retinal and RPE/choroidal tissues

Trial Number	Retina		RPE/Choroid	
	Left Eye	Right Eye	Left Eye	Right Eye
1	5.96	6.96	10.92	-
2	5.92	4.92	10.00	11.94
3	6.94	5.00	11.96	13.92
Average	6.27	5.63	10.96	8.62

Polarization shift measurements of combination retinal and RPE/choroidal tissues taken from the bovine left and right eyes are given in Table II.

Table II

Polarization shift (in degrees) for the combination of retinal and RPE/choroidal tissues

Trial Number	Retina & RPE/Choroid	
	Left Eye	Right Eye
1	11.92	15.92
2	11.2	14.94
3	13.96	16.92
Average	12.36	15.93

Variations in the polarization shifts between the left and right eyes may be due to

minuscule thickness differences in the prepared tissue samples, particularly, when different locations were chosen for collecting the data. The data from tables I and II suggest that the bovine ocular tissues are polarization dependent, and that the RPE/choroidal tissue shows a higher degree of polarization shift than the retina.

Polarization shifts in the bovine retina have also been measured at 24 hours of interval. During this period of time, the sample was kept refrigerated. It was found that the polarization shift decreases significantly after 48 hours after the sample preparation as shown in Table III below. A decrease in polarization shift may be attributed to the physiological degradation of the retinal tissue, thereby changing the its optical properties.

Table III

Polarization shift (in degrees) of bovine retinal tissue over time

Time(hrs)	Polarization Shift
0	6.96
24	5.92
48	2.10

Example 2

Retinal and RPE/choroidal tissues from the human healthy and diseased eyes (obtained from the National Disease Research Interchange) were carefully dissected and individually placed between a pair of glass slides separated by two cover slips or spacers at the two ends of the glass slides. Spacers may be used to prevent the glass slides from squeezing the tissues from its original, native shape to a compressed form. A small amount of vacuum grease was used in order to seal the open space between the glass slides so that the tissue was kept moist and retained in the space between the glass slides. These precautions may be taken in order to maintain the integrity of tissues' physiological properties, and also to make sure that the tissue optical properties do not change due to the compression and/or dehydration of the samples.

The data acquisition operation described herein was performed, and the polarization shift ($\Delta\theta$) and intensity measurements for healthy human retinal, RPE/choroidal, and combination retinal and RPE/choroidal tissues is shown in Table IV.

Table IV

Polarization shift (Δ θ) and intensity for healthy human ocular tissue

Eye	Trial Number	Retina		RPE/Choroid		Retina & RPE/Choroid	
		Δ θ	Intensity (mV)	Δ θ	Intensity (mV)	Δ θ	Intensity (mV)
Left	1	3.50	298.10	8.00	313.10	8.50	313.20
	2	3.00	308.60	6.50	322.50	7.50	312.40
	3	2.00	315.00	5.00	333.60	6.00	282.60
	AVG	2.83	307.23	6.5	323.07	7.33	302.73
Right	1	3.00	313.50	7.50	322.10	8.00	306.70
	2	2.80	310.20	6.50	266.40	6.50	319.10
	3	1.50	315.80	4.90	304.30	6.00	326.30
	AVG	2.43	313.17	6.30	297.60	6.83	317.37

The polarization shift (Δ θ) and intensity measurements for diseased human retinal, RPE/choroidal, and combination retinal and RPE/choroidal tissues is shown in Table V.

Table V

Polarization shift (Δ θ) and intensity for diseased human ocular tissue

Eye	Trial Number	Retina		RPE/Choroid		Retina & RPE/Choroid	
		Δ θ	Intensity (mV)	Δ θ	Intensity (mV)	Δ θ	Intensity (mV)
Left	1	6.50	2.61.90	11.00	259.25	12.50	210.70
	2	6.00	269.75	10.00	239.40	9.75	205.90
	3	5.00	289.50	8.50	218.70	7.50	201.50
	AVG	5.83	273.72	9.88	239.12	9.92	272.37
Right	1	6.50	227.25	10.00	249.10	10.00	230.10
	2	6.00	255.70	9.50	218.40	9.50	219.20
	3	4.9	301.80	8.50	208.90	7.50	208.90
	AVG	5.8	261.58	9.33	225.47	9.00	219.40

A comparison between the average polarization shifts (Δ θ) and average intensities between the diseased and healthy retinal, RPE/choroidal, and retinal and RPE/choroidal tissues (in stack) from the human left and right eyes is shown in table VI.

Table VI

Comparison between healthy and diseased human ocular tissue

Condition	Eye	Retina		RPE/Choroid		Retina & RPE/Choroid	
		Δ θ	Intensity (mV)	Δ θ	Intensity (I) (mV)	Δ θ	Intensity (I) (mV)
Healthy	Left	2.83	307.23	6.50	323.07	7.33	302.73
	Right	2.43	313.17	6.30	297.60	6.83	317.37
Diseased	Left	5.83	273.72	9.88	239.12	9.92	272.37
	Right	5.8	261.58	9.33	225.47	9.00	219.40

The data obtained shows that there is a substantial increase in the polarization shift for diseased ocular tissues (retina and RPE/choroid), compared to that of healthy tissues. The diseased eyes had been previously frozen, and were thawed before preparing the tissue samples for polarization measurements. The normal eyes had not been frozen. Based on previous observations of bovine retinal tissue at different times after freezing (see example 1, table III) one of ordinary skill in the art would infer that the polarization shift would have been even more pronounced in fresh diseased tissues.

Further, the polarization shift in human retinal tissue at 24 hours intervals was measured and is shown in table VII.

Table VII

Polarization shift (in degrees) of human retinal tissue over time

Time (hrs)	Polarization Shift (degrees)
0	13.0
24	11.5
48	5.0

It can be observed from table VII that the polarization change decreases significantly after 48 hours after sample preparation. During this period of time, the sample was kept refrigerated. The decrease in polarization shift can be attributed to the physiological degradation of the tissue, thereby changing the optical properties.

The observed variations in the polarization shifts between left and right eyes could be due to the minuscule differences in thickness of the tissue samples, particularly, when different locations were chosen to take the measurements. The RPE/choroidal tissue shows a higher degree of polarization shift than the retina tissue. The shift in the combined retinal and RPE/choroidal tissues are substantially higher. From the tables IV-VII, it can be determined that the higher the polarization shift, the lower the intensity of the scattered polarized light.

The invention may include method and/or apparatus for assisting in the non-invasive diagnosis and treatment of diabetic retinopathy, macular degeneration, and other ocular diseases, including cancer detection.

The appended claims are not to be interpreted as including means-plus-function limitations, unless such a limitation is explicitly recited in a given claim using the phrase(s) "means for" and/or "step for." Subgeneric embodiments of the invention are delineated by the

appended independent claims and their equivalents. Specific embodiments of the invention are differentiated by the appended dependent claims and their equivalents.

The terms "a" or "an", as used herein, are defined as one or more than one. The terms "including" and/or "having", as used herein, are defined as comprising (i.e., open language). The term "coupled", as used herein, is defined as connected, although not necessarily directly, and not necessarily mechanically. The term "approximately", as used herein, is defined as at least close to a given value (e.g., preferably within 10% of, more preferably within 1% of, and most preferably within 0.1% of). The term "substantially", as used herein, is defined as at least approaching a given state (e.g., preferably within 10% of, more preferably within 1% of, and most preferably within 0.1% of).

CLAIMS

We claim:

1. A method for diagnosing an ocular disease, comprising:
placing an ocular tissue in the path of a light beam;
measuring a polarization shift of the light beam; and
diagnosing an ocular disease if the measured polarization shift corresponds to a polarization shift of a neovascularized tissue.

2. The method of claim 1, wherein the ocular tissue includes a retinal tissue.

3. The method of claim 1, wherein the ocular tissue includes an RPE/choroidal tissue.

4. The method of claim 1, wherein the light includes a laser.

5. The method of claim 1, wherein the ocular disease includes diabetic retinopathy.

6. The method of claim 1, wherein the ocular disease includes macular degeneration.

7. The method of claim 1, wherein the ocular disease includes cancer.

8. A method for diagnosing an ocular disease, comprising:

placing an ocular tissue in the path of a light beam;

measuring an intensity variation of the light beam; and

diagnosing an ocular disease if the measured intensity variation corresponds to the intensity variation of a neovascularized tissue.

9. The method of claim 8, wherein the ocular tissue includes a retinal tissue.

10. The method of claim 8, wherein the ocular tissue includes an RPE/choroidal tissue.

11. The method of claim 8, wherein the light includes a laser.

12. The method of claim 8, wherein the ocular disease includes diabetic retinopathy.

13. The method of claim 8, wherein the ocular disease includes macular degeneration.

14. The method of claim 8, wherein the ocular disease includes cancer.

15. A method for diagnosing an ocular disease, comprising:

placing an ocular tissue in the path of a light beam;

measuring a polarization shift of the light beam;

measuring an intensity variation of the light beam; and

diagnosing an ocular disease if the measured polarization shift and intensity variation correspond to a polarization shift and intensity variation of a neovascularized tissue.

16. An apparatus for diagnosing an ocular disease, comprising:

a laser;

a polarizer coupled to the laser;

a tissue sample holder coupled to the polarizer, the tissue sample holder having an ocular tissue sample;

an analyzer coupled to the tissue sample holder;

a detector coupled to the analyzer; and

a data acquisition system coupled to the detector, the data acquisition system measuring a polarization shift of the light beam and diagnosing an ocular disease if the measured polarization shift corresponds to a polarization shift of a neovascularized tissue.

17. The apparatus of claim 16, wherein detector includes a photodiode.

18. The apparatus of claim 16, wherein the data acquisition system includes a digital

meter.

19. The apparatus of claim 16, wherein the data acquisition system includes a computer.

20. The apparatus of claim 16, further comprising a power supply coupled to the detector.

ABSTRACT OF THE DISCLOSURE

A method for diagnosing an ocular disease includes placing an ocular tissue in the path of a light beam, measuring a polarization shift of the light beam, and diagnosing an ocular disease if the measured polarization shift corresponds to a polarization shift of a neovascularized tissue. An apparatus for diagnosing an ocular disease includes a laser, a polarizer coupled to the laser, a tissue sample holder coupled to the polarizer, the tissue sample holder having an ocular tissue sample, an analyzer coupled to the tissue sample holder, a detector coupled to the analyzer, and a data acquisition system coupled to the detector, the data acquisition system measuring a polarization shift of the light beam and diagnosing an ocular disease if the measured polarization shift corresponds to a polarization shift of a neovascularized tissue.

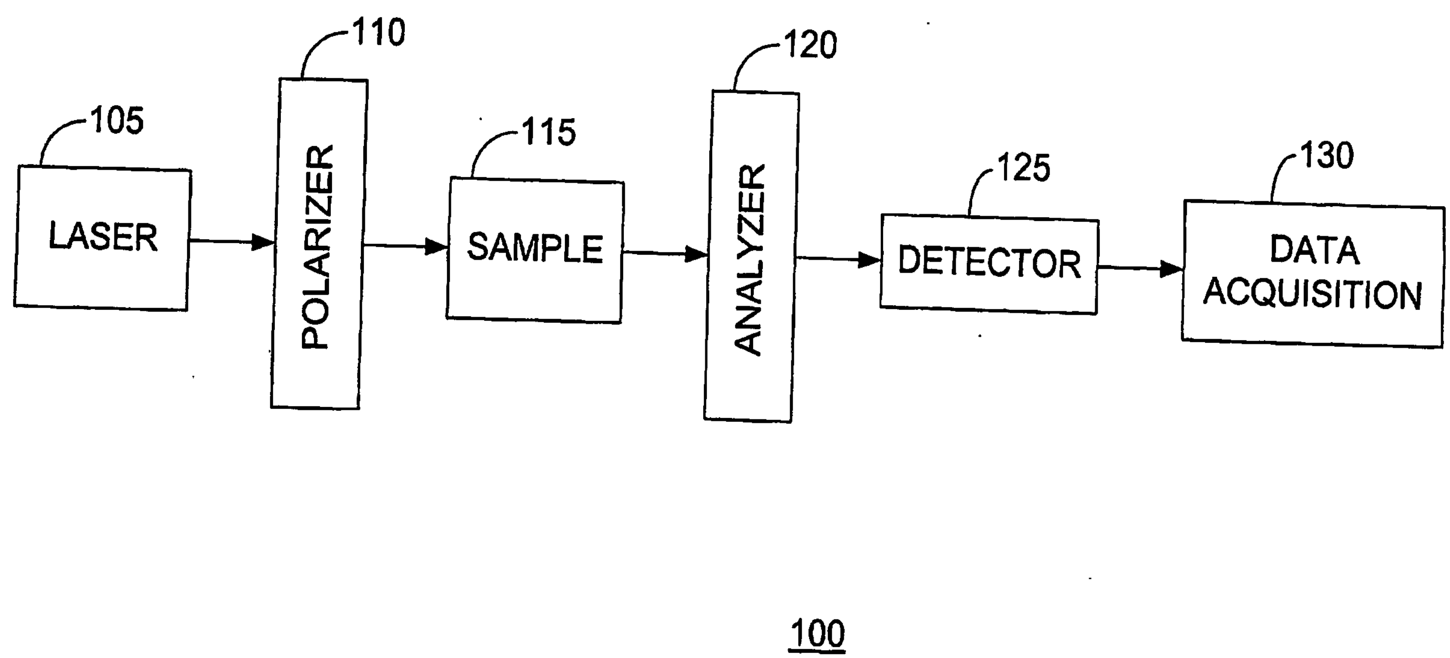
105
LASER
110
POLARIZER
115
SAMPLE
120
ANALYZER
125
DETECTOR
130
DATA
ACQUISITION
100

FIG. 1

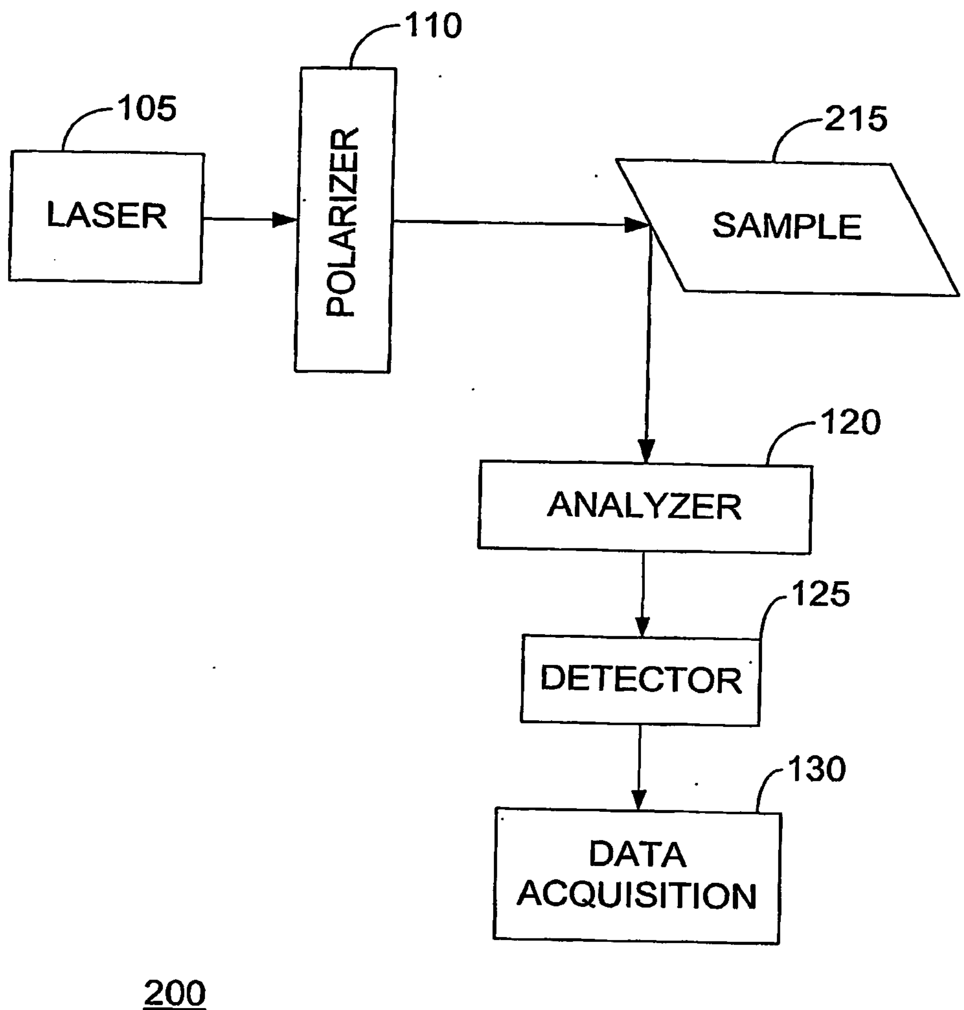
110
105
215
LASER
POLARIZER
SAMPLE
120
ANALYZER
125
DETECTOR
130
DATA
ACQUISITION
200

FIG. 2